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The Effects of Cold Acclimation on Calmodulin in the crayfish, Procambarus clarkii

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The Effects of Cold Acclimation on Calmodulin in the crayfish, *Procambarus clarkii*

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Abstract

Homeostasis of intracellular calcium is important because calcium acts as a second messenger in many cellular processes, and to maintain this homeostasis there are many forms of transport [1]. The Plasma Membrane Ca^{2+}-ATPase (PMCA) actively removes Ca^{2+} from the cell and is activated by the protein calmodulin (CaM) [4]. Exposure to cold ambient temperatures causes an influx of calcium into cells, and thus can be used to study calcium regulatory proteins such as CaM. Previous studies have seen an increase in expression of PMCA in the cold [1]. We predicted a similar increase in CaM due to its role as a PMCA activator. After two weeks of cold acclimation, the expression of CaM was measured by Real-Time PCR in five tissues. No significant difference in CaM expression was measured between cold and room temperature acclimated animals, although there was a trend towards a small increase in expression, especially in the antennal gland.

Introduction

The freshwater crayfish, *Procambarus clarkii,* has been previously used to study the regulation of genes involved in calcium transport because of its periodic molt cycle, during which large amounts of calcium are transported.

Calmodulin (CaM) is a Ca^{2+} binding protein that is part of the Ca^{2+} second-messenger system and is responsible for controlling many processes within the cell, including activation of the Plasma Membrane Ca^{2+}-ATPase [PMCA, 2]. PMCA actively transports calcium against its electrochemical gradient out of the cell in order to maintain a low intracellular Ca^{2+} concentration.

Cold acclimation disrupts Ca^{2+} homeostasis by increasing the open probability of Ca^{2+} channels. An increase in concentration of Ca^{2+} elicits an increase in Ca^{2+} export and therefore an increased expression of Ca^{2+} exporting protein-encoding genes like PMCA. The expression of PMCA has been shown to increase during periods of higher Ca^{2+} transport (during postmolt and cold acclimation) [3] [1]. **Thus, we hypothesize that the expression of CaM will increase during cold acclimation due to its role in the regulation of PMCA.**

Methods

Freshwater crayfish were obtained from Niles Biological and acclimated for two weeks in room temperature tanks at 23˚ C. Half of the crayfish were randomly selected and moved to cold temperature (4˚ C) and after 7 days the crayfish were decerebated and dissected; removing the hepatopancreas (liver), the gills, the antennal glands (kidney), the axial tail muscle and the cardiac muscle. The tissues were kept at -80˚ C, RNA STAT-60 was used to isolate the total RNA and DNAase-free TURBO removed impurities from the tissues. Applied Biosystems Reverse Transcriptase Kit converted the RNA into cDNA. The amount of cDNA was quantified with real-time PCR using the ΔΔCt method (Figure 1), amplifying the CaM gene and an internal calibrator, 18S. The fold difference in expression (RQ) was calculated by the equation $RQ=2^{-\Delta\Delta Ct}$ for each sample. The ΔCt value for a cold individual was then subtracted from the average ΔCt for the control (room temperature) to find the value of the ΔΔCt. The ΔΔCt was then used to find the RQ value, or the fold change in expression, by $RQ=2^{-\Delta\Delta Ct}$. The RQ value can then be used to compare the two groups.

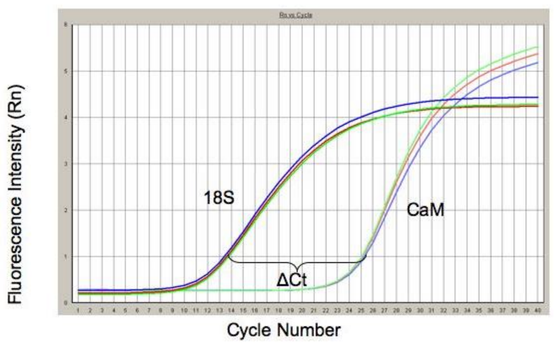

Figure 1. Amplification plot of real-time PCR using antennal gland cDNA and calmodulin primers and 18S rRNA primers. Samples were run in triplicate.

Results

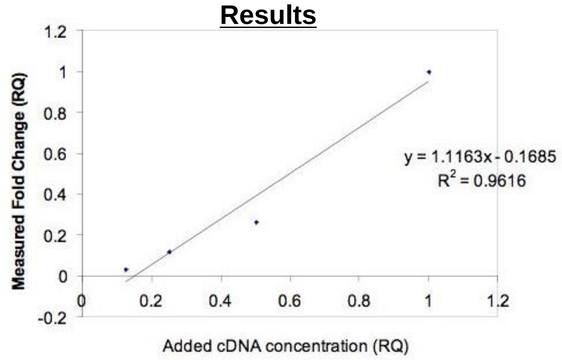

Figure 2. Determination of primer efficiency; cDNAs of different concentrations were amplified.

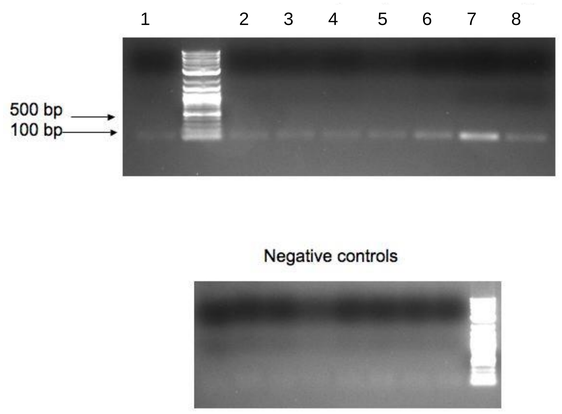

Figure 3. Agarose gel of selected RT-PCR products.of liver tissues. A single band of a predicted size (100 bp) is present in lanes 1-8. No bands are in the negative controls. 1% agarose, visualized by EtBr.

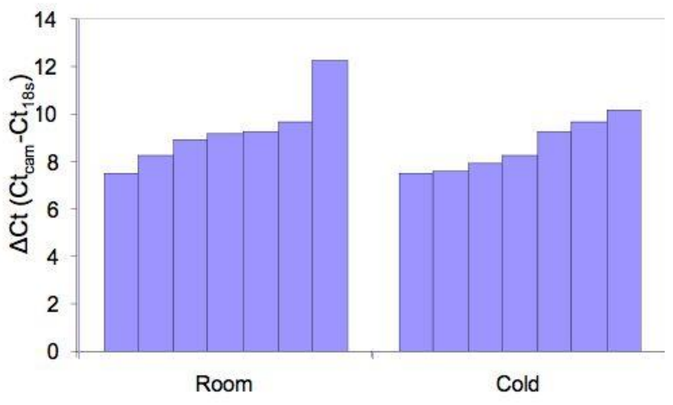

Figure 4. Effect of cold acclimation on the antennal gland expression of calmodulin in the crayfish *P. clarkii* using real-time PCR. ΔCt is the difference between the 18S and the CaM in the amount of cycles until the expression crosses a threshold value; a small ΔCt indicates a larger expression level. Each bar indicates an individual crayfish.

Results

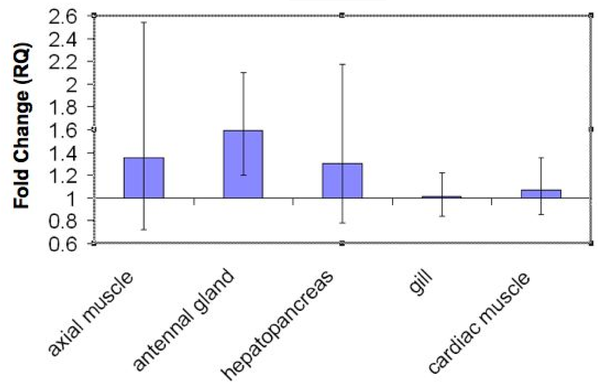

Figure 5. RQ values for the fold change in expression of calmodulin in cold-acclimated tissues relative to average in the room temperature in the crayfish *P. clarkii*. Bars are +/- SE. N=7 for antennal, liver and gill tissues; N=6 for heart; N=5 for tail. No significant difference between cold and room (t-test, $p > .05$). RQ is equal to $2^{-\Delta\Delta Ct}$.

Discussion

•The primers were tested to ensure correct amplification by varying the concentration of the cDNA (Figure 2).

•A product of the correct size was amplified by our calmodulin primers, as seen in the liver tissues in Figure 3. Both the primers and the cDNA were functioning correctly.

•Individual crayfish demonstrated some variability in the expression of CaM, in both room and cold tissues (Figure 4).

•The effect of cold acclimation on the expression of the gene calmodulin was found to be not significant in *P. clarkii* (Figure 5) although a slight increase in cold acclimated tissue was noted. Further studies could use a larger sample size to potentially see a more noticeable change.

Acknowledgements

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